EXAMPLE 8

D-RTR Tetramer Inhibition of N-acetyl-PGP or N-methyl-PGP Induced PMN Polarization

The RTR complementary peptide has been shown to inhibit the polarization of polymorphonuclear leukocytes activated by N-acetyl-PGP. The complementary sequence, RTR, was designed to specifically interact hydropathically with the PGP sequence in N-acetyl-PGP and, therefore, should also interact with the same sequence in N-methyl-PGP. The D-RTR tetrameric peptide was designed to inhibit N-acetyl-PGP or N-methyl-PGP induced polymorphonuclear leukocyte polarization, but have a greater stability *in vivo* by resisting proteolytic degradation.

A preliminary study showed that the D-RTR tetramer inhibited (mean ± SD) 800 μM N-acetyl-PGP induced polymorphonuclear leukocyte polarization as follows: 100 nM D-RTR tetramer = 37% ± 35% inhibition (n=7), 1 μM D-RTR tetramer = 65% ± 26% inhibition (n=6) and 10 μM D-RTR tetramer = 92% ± 6% inhibition (n=6). The D-RTR tetramer inhibited (mean ± SD) 1 mM N-

methyl-PGP induced polymorphonuclear leukocyte polarization as follows: 1-10 μM D-RTR tetramer = 14% ± 10% inhibition (n=5), 40-100 μM D-RTR tetramer = 45% ± 7% inhibition (n=2) and 200-800 μM D-RTR tetramer = 100% inhibition (n=5).

EXAMPLE 9

Results

All four complementary (antisense) peptides, containing the RTR sequence, showed substantial inhibition of N-acetyl-PGP activated polymorphonuclear leukocyte polarization (Table 1). The RTR tetrameric peptide was a powerful inhibitor of N-acetyl-PGP (ID_{50} of 200 nM). The RTR dimer was much less potent (ID_{50} of 105 μM). Both monomers, RTR (ID_{50} of 2.5 mM) and RTRGG (ID_{50} of 2.1 mM), were only antagonistic at millimolar concentrations. Preincubation of the RTR tetrameric peptide with N-acetyl-PGP or neutrophils for 5 min did not change the results described above. An additional antisense peptide, ASA tetramer, failed to show any inhibition of polymorphonuclear leukocytes activated by N-acetyl-PGP.

TABLE I

Complementary Peptide Inhibition of N-acetyl-PGP Activated PMN Polarization

Complementary Peptides	Antagonist Activity (ID_{50})	p-value
RTR tetramer	200 nM ± 75 nM	<0.001
RTR dimer	105 μM ± 68 μM	0.001
RTR monomer	2.5 mM ± 1.2 mM	<0.001
RTRGG monomer	2.1 mM ± 0.8 mM	<0.001
ASA tetramer	None, ≤ 4 mM	--------

* Untreated PMNs (negative control) produced a polarization response of 7.8% ± 4.4% (n = 41). PMNs activated with 500 μM N-acetyl-PGP (positive control) produced a polarization response of 56.5% ± 16.4% (n = 41). This chemoattractant concentration was selected from the linear portion of the dose response curve, yielding approximately 50% polarization after subtraction of the negative control values. Antagonistic activity (ID_{50}, mean ± standard deviation) was interpolated from five dose response curves for each complementary peptide.